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Prevalence of Gentamicin- and Amikacin-Resistant Bacteria in Sink Drains

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Sink drains from the Veterans Administration Medical Center, University of Oklahoma Health Sciences Center, and the Oklahoma City community were selectively cultured for gentamicin- and amikacin-resistant bacteria. Aminoglycoside-resistant organisms were found in 86% (Veterans Administration Medical Center, 88%; University of Oklahoma Health Sciences Center, 88%; and Oklahoma City community, 77%) of all 233 sink drains sampled. Of 207 sink drains harboring aminoglycoside-resistant organisms, 99% of the organisms were gentamicin resistant and 82% were amikacin resistant. These data suggest that aminoglycoside-resistant organisms are commonly present in the environment.

Hospital sink drains have been shown to harbor gentamicin-resistant bacteria which can be transferred to hands during washing (4). After contamination, hands of hospital personnel can be reservoirs for the transmission of bacteria to patients (3, 11, 19). In addition, several outbreaks of serious infections, caused by gentamicin-resistant organisms, have been reported (2, 7). To gain additional information on reservoirs of antibiotic-resistant organisms, we decided to further investigate the occurrence of bacteria in sink drains. The purpose of this investigation was to determine the following: (i) the length of survival of various bacteria in wet and dry environments, (ii) the identity and prevalence of gentamicin- and amikacin-resistant bacteria in sink drains, and (iii) the occurrence of clinical isolates which might be coming from sink drains.

MATERIALS AND METHODS

Organisms. All *Enterobacteriaceae* and gram-positive cocci were identified by conventional methods. Non-*Enterobacteriaceae*, gram-negative isolates were termed nonfermenters and identified by conventional (9, 16, 21, 22) methods and the API 20E System (Analytab Products).

Viability studies. Bacterial suspensions were made in sterile deionized water, pH 6.7, to a turbidity equal to that of a 0.5 MacFarland standard (25 to 30 Klett-Summerson colorimeter units). The suspensions contained approximately 10^7 to 10^8 colony-forming units per ml. Sterile, 5-mm-diameter, glass beads were placed into each homogeneous suspension and allowed to stand for 5 min. The beads were then removed and placed in a sterile glass petri dish to air dry, which took approximately 5 min. Immediately after drying, a bead was placed in 1.0 ml of Trypticase soy broth (BBL Microbiology Systems) as a positive control. The remaining beads were placed in a sterile test tube, and one was inoculated periodically into 1.0 ml of Trypticase soy broth for testing viability from a dry environment. For study of survival in a wet environment, a loopful (approximately 0.05 ml) of the previously mentioned suspensions was inoculated periodically into 1.0 ml of Trypticase soy broth as a viability check. Additional cultures were made on Mueller-Hinton agar plates for both wet- and dry-environment studies observing for characteristic colonial morphology to rule out contamination. The cultures were incubated for 48 h at 25 to 35°C. Relative humidity in the test laboratory was 38%.

Test sites for sink drain studies. Sink drain cultures were taken at the Veterans Administration Medical Center, University of Oklahoma Health Sciences Center, and the Oklahoma City community. Oklahoma Health Sciences Center cultures were from nonhospital sites, including the Library, Biomedical Sciences Building, and the Basic Sciences Building. Oklahoma City community cultures were taken from private homes, service stations, and shopping centers.

Sink drain prevalence studies. Sink drains were sampled, as described previously (5), by passing a sterile, cotton-tipped applicator stick through the sink strainer after a tap water rinse period. The sampled material was then plated onto a biplate containing deoxycholate agar with gentamicin (Schering Corp.; 10 μg/ml) on one side and amikacin (Bristol Laboratories; 35 μg/ml) on the other. Antibiotics were incorporated into molten medium (50°C) before solidification. The above-mentioned concentrations were chosen because serum gentamicin levels of greater than 10 μg/ml and amikacin levels of greater than 35 μg/ml are considered toxic; therefore, organisms which can grow at these concentrations are resistant for practical purposes. This antibiotic-containing deoxycholate agar only allowed for the growth of gentamicin- or amikacin-resistant gram-negative organisms.

Special study on a test sink. The sink drain and tap water samples from a sink in the Veterans Administration Medical Center microbiology laboratory were cultured over a 14-day period. This sink was not exposed to clinical isolates. Qualitative water cultures were determined as described elsewhere (15).

History survey of clinical isolates. Data concerning clinical nonfermenting isolates were collected from the Veterans Administration Medical Center microbiology section logbooks. Results concerning amikacin resistance in clinical isolates were based on disk agar diffusion testing with a 10-μg, commercially prepared (Difco Laboratories) amikacin disk.

RESULTS

Viability studies. Results on the survival of organisms in wet and dry environments are shown in Table 1. In addition, the following isolates (with their respective numbers given within parentheses) were shown to survive in deionized water: clinical isolates surviving for more than 210 days—*Klebsiella pneumoniae* (27), *Proteus morganii* (9), *Proteus rettgeri* (10), *Proteus vulgaris* (8), *Pseudomonas aeruginosa* (28), and *Serratia marcescens* (27); and sink drain isolates surviving for more than 180 days—*Achromobacter* spp., (22), *Acinetobacter* spp., (8), *Aeromonas* spp., (1), *Enterobacter* spp., (2), *Moraxella* spp., (5), *Flavobacterium* spp., (12), *Pseudomonas acidovorans* (2), *P. aeruginosa* (11), *Pseudomonas cepacia* (25), *Pseudomonas fluorescens* (2), *Pseudomonas maltophilia* (73), *Pseudomonas putida* (3), *Pseudomonas* spp., (45) and *Pseudomonas stutzeri* (5). Generally, all organisms survived better in a wet environment than in a dry one. The gram-positive organisms survived better than most of the gram-negative organisms in a dry atmosphere, but the gram-negative organisms survived better than the gram-positive organisms in a moist setting. The *Candida albicans* strains were only viable for 1 day in a dry atmosphere; they were not tested in a wet environment because the fungi are known to survive for long periods in water (1, 12).

Sink drain prevalence studies. Since selective media were used, results of this study reflect only on the occurrence of gentamicin- and amikacin-resistant gram-negative organisms. Table 2 shows the results of sink drain cultures. At the Veterans Administration Medical Center, 170 sink drains were cultured, and of these, 149 contained aminoglycoside-resistant organisms. All of the 149 sink drains had gentamicin-resistant organisms, but only 131 had amikacin-resistant organisms. Of the 149 sink drains with aminoglycoside-resistant organisms, 180 different isolates were encountered. From 170 sink drains cultured at the Veterans Administration Medical Center, 88% had bacteria which were resistant to gentamicin, amikacin, or both. Cultures yielded a variety of organisms, mostly nonfermenting gram-negative bacilli (Table 3). Among the most common gentamicin- or amikacin-resistant sink drain isolates at the Veterans Administration Medical Center, University of Oklahoma Health Sciences Center, and Oklahoma City community were *P. maltophilia*, *P. cepacia*, and other *Pseudomonas* spp. Sink drains harboring two to three different organisms were not uncommon. Gentamicin-resistant bacteria were more prevalent than amikacin-resistant isolates. Few *Enterobacteriaceae* organisms were found, and the absence of *P. aeruginosa*

TABLE 1. *Viability of organisms in dry and wet environments*

Organism (no. tested)	Viability (days)			
	Dry		Wet	
	Mean	Range	Mean	Range
Acinetobacter spp. (5)	7	7–8	>250	180–>250
Citrobacter spp. (10)	<1	0–1	>250	
Enterobacter spp. (10)	5	3–7	>250	
Escherichia coli (12)	2	0–3	>250	180–>250
Klebsiella pneumoniae (10)	4	2–5	>250	
Proteus mirabilis (10)	1	1–2	>250	
Proteus spp., other (10)	<1	0–1	>250	180–>250
Pseudomonas aeruginosa (20)	<1	0–1	>250	
Serratia marcescens (10)	1	0–2	>250	
Staphylococcus aureus (20)	8	7–9	>180	180–>250
S. epidermidis (10)	9	8–10	110	80–120
Streptococcus, group A (5)	5	5–6	22	19–24
Streptococcus, group B (5)	6	5–7	22	20–23
Streptococcus, group D enterococci (10)	9	8–11	93	90–110
Candida albicans (10)	1	1–1		

TABLE 2. *Sink drain cultures*

Area cultured[a]	Total no. of sinks cultured	No. with organisms resistant to the following:		
		Aminoglycoside	Gentamicin	Amikacin
VAMC	170	149 (88)[b]	149 (88)	131 (77)
OUHSC	33	29 (88)	29 (88)	24 (73)
OCC	30	23 (77)	23 (77)	13 (43)
Total	233	201 (86)	201 (86)	168 (72)

[a] VAMC, Veterans Administration Medical Center; OUHSC, University of Oklahoma Health Sciences Center; OCC, Oklahoma City community.

[b] Percentage of total sinks cultured.

TABLE 3. *Sink drain isolates*

Organism	VAMC[a]			OUHSC[a]			OCC[a]		
	No. (%)[b]	GM[c]	AN[c]	No. (%)[b]	GM[c]	AN[c]	No. (%)[b]	GM[c]	AN[c]
Achromobacter spp.	23 (12.8)	91	57	0			0		
Acinetobacter anitratus	4 (2.2)	75	75	1 (1.9)	100	100	5 (17.2)	100	100
A. lwoffi	1 (0.6)	100	0	3 (5.8)	67	67	0		
Aeromonas spp.	1 (0.6)	100	100	1 (1.9)	100	0	0		
Enterobacter agglomerans	1 (0.6)	100	0	0			0		
E. cloacae	1 (0.6)	100	0	0			0		
Flavobacterium spp.	8 (4.4)	50	88	5 (9.6)	40	100	5 (17.2)	80	60
Moraxella spp.	5 (2.7)	100	40	2 (3.8)	100	50	2 (6.9)	100	50
Pseudomonas acidovorans	0			2 (3.8)	100	0	0		
P. aeruginosa	14 (7.8)	100	0	0			0		
P. cepacia	18 (10)	89	67	9 (17.3)	78	67	2 (6.9)	100	0
P. fluorescens	1 (0.6)	100	100	2 (3.8)	100	0	2 (6.9)	100	100
P. maltophilia	58 (32)	79	71	16 (30.8)	94	94	5 (17.2)	80	60
P. putida	3 (1.7)	67	33	0			1 (3.4)	100	0
Pseudomonas spp.	37 (20.5)	95	51	11 (21.2)	91	27	6 (20.7)	100	67
P. stutzeri	5 (2.7)	100	0	0			1 (3.4)	100	0
Total	180	87	76	52	88	73	29	93	52

[a] VAMC, Veterans Administration Medical Center, OUHSC, University of Oklahoma Health Sciences Center; OCC, Oklahoma City community.
[b] Percentage of total isolates from a given area.
[c] Percentage of isolates resistant to gentamicin (GM) or amikacin (AN).

isolates in the University of Oklahoma Health Sciences Center and Oklahoma City community was also striking.

A separate study, involving sink drain and qualitative tap water cultures on a single laboratory sink, was also done over a 14-day period. Sink drain cultures revealed two to four different organisms present, from which the same group of organisms persisted from day to day. Daily tap water cultures were all negative for growth, except one, which yielded *Flavobacterium* spp., which was never a sink drain isolate from the test sink during the test period.

Historical survey of clinical isolates. The number of clinical isolates of nonfermenting bacilli at the Veterans Administration Medical Center from January 1978 to June 1979 is noted in Table 4. Of 497 *P. aeruginosa* isolates, 84 were gentamicin resistant and 65 were amikacin resistant. A significant percentage of *P. maltophilia* isolates was also gentamicin and amikacin resistant. During a 4-year period (1976 to 1979) at the Veterans Administration Medical Center, no significant correlation could be found between aminoglycoside usage and resistance. Data are shown in Table 5 for gentamicin and were similar for tobramycin and amikacin. The number of pharmacy units issued should be closely related to the actual usage of gentamicin.

TABLE 4. *Clinical isolates of nonfermenters at Veterans Administration Medical Center (January 1978 to June 1979)*

Organism (no. isolated)	Resistance (%) to:	
	Gentamicin	Amikacin
Achromobacter spp. (1)	0	0
Acinetobacter anitratus (28)	14	7
A. lwoffi (9)	11	11
Aeromonas hydrophila (2)	0	0
Alcaligenes spp. (3)	0	0
Flavobacterium spp. (3)	33	67
Moraxella spp. (4)	0	0
Pseudomonas aeruginosa (497)	17	13
P. cepacia (1)	100	100
P. fluorescens (7)	0	14
P. maltophilia (12)	58	75
Pseudomonas spp. (23)	13	9
Total (590)	17	14

DISCUSSION

The potential role of hospital sink drains in nosocomial infections can become even more meaningful when one considers antibiotic-resistant sink drain bacteria. Sink drains harbor gentamicin-resistant bacteria more consistently than do other sites (dry surface areas, e.g., walls, floors, and beds) in patient care areas (5). The main difference between sink drains and these other sites is the presence of water. Water can affect the presence of antibiotic-resistant bacteria by increasing their survival. Previous studies have dealt with organism survival in wet and dry states (1, 3, 4, 12, 14, 17); however, we failed to find a study comparing many organisms

TABLE 5. *Gentamicin usage versus susceptibility of clinical isolates*

Organism	Susceptibility (%) by year[a]			
	1976	1977	1978	1979
Escherichia coli	94	96	94	92
Enterobacter spp.	91	85	89	89
Klebsiella pneumoniae	87	93	90	92
Proteus mirabilis	94	94	90	84
Proteus spp., other	70	89	71	71
Pseudomonas aeruginosa	85	86	82	85
Serratia spp.	50	45	58	37

[a] Pharmacy units (80 mg) issued: 1976, 10,633; 1977, 13,065; 1978, 13,807; 1979, 12,057.

which would be commonly seen as clinical or environmental isolates. The long survival of gram-negative bacteria reported here helps to explain their common occurrence in sink drains. It is also of interest to note that stock cultures of many organisms can be kept in sterile, deionized water for months, thus saving considerable time, effort, and expense in hospital and university laboratories, where monthly stock culture transfers often take place.

In 1977, Moellering et al. reported on aminoglycoside-resistant clinical isolates from 1974 to 1976 at Massachusetts General Hospital (13). The five most common nonfermenters were *Acinetobacter calcoaceticus* subsp. *anitratus* (*Herellea vaginicoli*), *P. aeruginosa*, *P. maltophilia*, *Flavobacterium* spp., and *Pseudomonas* spp. The top five nonfermenters at the Veterans Administration Medical Center included all of these except *Flavobacterium* spp. During the period from January 1978 to June 1979, 106 clinical isolates of aminoglycoside-resistant organisms were reported. If these were all nosocomially related and originated from sink drains, they would represent a significant number. Our data show that common clinical isolates are not necessarily common sink drain isolates, however. Indeed, it is possible that the apparent lack of sink drain flora as clinical isolates shows they are not epidemiologically important. We suggest that some sink drain isolates may be nosocomially related.

Typical hospital sink drains apparently harbor one to two aminoglycoside-resistant nonfermenting organisms which reside in these moist areas. This study shows that sink drain isolates remain viable for many months in water. These organisms can pass to compromised patients from employees via hand washing (3, 5, 11, 19), or they can transfer their resistance factors to other organisms which may be pathogens (10). This route of nosocomial infection does not appear to represent a large percentage of nosocomial infections, but could become more important in situations such as hospital outbreaks. In these emergency situations, antiseptic agents should be used for hand washing (20), and sink drains should be disinfected daily (5). Aminoglycoside-resistant nonfermenting organisms should be strongly considered as nosocomial pathogens when isolated in clinical microbiology laboratories.

These antibiotic-resistant sink drain flora could be originating from tap water, carried into hospitals by visitors and personnel, or simply ubiquitous, especially to the sewer system. The resistance of *Pseudomonas* spp. may arise from non-antibiotic-mediated mechanisms, such as lack of permeability to antibiotics, which might explain the apparent ubiquitous aminoglycoside resistance of these organisms. We feel that their resistance is inherent and not the result of antibiotic usage.

Evidence is also available to indicate that selective pressure by heavy antibiotic usage may not be responsible for all common aminoglycoside resistance in bacteria. Gentamicin was used for 7 years at one hospital before resistant strains of *Klebsiella* were seen (18). In addition, Gardner et al. reported the presence of aminoglycoside-resistant transfer factors from bacteria isolated in the antibiotic-virgin Solomon Islands (6). After inoculation, antibiotic-resistant organisms were also found to multiply and persist in the gastrointestinal tract of poultry in the absence of selective pressure by antibiotics (8). Our study notes the common presence of aminoglycoside-resistant, nonfermenting gram-negative bacilli in sink drains both inside and outside the hospital environment. More investigations are needed to determine the occurrence of sink drain flora as nosocomial pathogens.

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